Remarks

Claims 1-20 are currently pending in this application. Claims 9, 10, and 15-18 stand withdrawn. Claims 1-8, 11-14, and 19-20 are under examination and stand rejected or objected to.

Claims 1-8 and have been amended to further clarify what is recited in the claims. Claims 11-14 have been amended to overcome the Office's rejections. New claim 21, which recites a range limitation originally recited in claim 2, has been added. New claim 22, which recites the use of classes of alcohols originally recited in claim 4, has been added. Support for the amendments is found in the as-filed application, and no new matter has been added.

The specification has also been amended to clarify the written description and to add an appropriate abstract. Again, no new matter has been added.

Abstract

The Office has required the inclusion of a one paragraph abstract. In response, Applicants have provided on the last page of the substitute specification included in this response a one paragraph abstract. This abstract is a revised version of the "summary" section included on the last page of the original as-filed specification, and is now appropriately titled "Abstract." As with the submitted versions of the substitute specification, Applicants have provided both a markup and clean version of the abstract.

Specification

The Office has requested that Applicants correct any errors in the specification. In response, Applicants have made a number of amendments to the specification, to correct errors and to clarify the specification. These amendments are included in a substitute specification submitted with this response, in both markup and clean versions.

The amendments to the specification include (1) correction of grammatical errors, (2) the addition of organizational headings corresponding to the specification section requirements of 37 CFR 1.77, (3) the addition of paragraph numbers, (4) the movement of paragraphs to the appropriate section (particularly the movement of the Brief Description of the Drawings paragraphs from their original location toward the end of the Detailed Description to the appropriate section after the Summary of the Invention), (5) the occasional deletion or addition of

words and phrases to add clarity to the text, and (6) the addition or substitution of "alcoholysis" in a number of places for "hydrolysis."

In the art, "hydrolysis" refers to the production of the fatty acid itself from a fat or oil (along with glycerol) by reaction with water. "Alcoholysis" is more properly used when referring to transesterification reactions wherein a new fatty acid ester is formed from the reaction of oils or fats with an alcohol. The improper use of the two words in the specification (and in the claims, as discussed later) was apparently result of a translation error. Both processes are designated in the original German language application as "spaltung," which means "cleavage." "Spaltung" can refer to either hydrolysis or alcoholysis, depending on the context. The difference between "hydrolysis" and "alcoholysis" is well established in the art, and the skilled artisan would recognize this substitution as being appropriate in the context of the reactions as described. No new matter has been added in the specification amendments.

35 USC § 112 (second paragraph)

The Office has rejected claim 2 as being indefinite. Specifically, the office alleged that the language ". . . alcohol is used in an excess of 2 to 100%" recites a range above a maximum possible amount of alcohol and includes an amount of alcohol that would kill the lipase enzyme.

In response, Applicants have amended claim 2 to clarify the amount of alcohol recited. Claim 2 as amended now recites an amount alcohol used that "is an excess of from 2 to 100%, as compared to the amount of alcohol stoichiometrically necessary for complete esterification." Thus the recited range is determined not as a percentage of the reaction mixture, but as the percentage range by which the moles of alcohol present exceeds the moles needed to exactly use up and convert the fat or oil reactants to a different ester product, assuming 100% yield ("stoichiometrically necessary").

For example, exactly one mole of a triglyceride fat will require three moles of a typical n-alcohol to completely esterify to a different ester product (so three moles alcohol are "stoichiometrically necessary"). If three moles of the n-alcohol are added to one mole of triglyceride, there is no excess alcohol. However, if four moles of alcohol are added to one mole of triglyceride, there is a 33% excess of alcohol (1mole excess/3 moles stoichiometrically necessary), which is within the claimed range. Thus, a mixture of well over 100% excess alcohol is allowed (i.e. in the above example, seven moles alcohol and one mole triglyceride would result in four moles, or 133%, excess alcohol).

The specification fully supports the claim as amended. For example, the original language of paragraph [0023] stated that the alcohols "can be added in the stoichiometrically required proportion for ester formation (emphasis added). The paragraph continues by stating that it would however be advantageous to use "an excess of 2% to 100%, preferably 5 to 20%, based on the stoichiometric requirements" of alcohol (emphasis added). Alcohol excess percentages are used in the same way in other parts of the specification. See e.g. paragraphs [0072] and [0078] (teaching a 10% stoichiometric excess of alcohol).

In the context of the original paragraphs, the claimed range clearly refers to the percentage by which the alcohol exceeds the "stoichiometrically required amount," and does not refer to an absolute range. Without adding new matter, Applicants have amended both paragraph [0023] and claim 2 to further clarify that this is what is meant by the claimed range of excess alcohol. In view of the clarifying amendments and the support for the amendments found in the specification, Applicants respectfully request that the Office reconsider and withdraw its indefiniteness rejection of claim 2.

Amendment to Claim 3

Applicants have amended claim 3 to more clearly recite what is meant by "based upon" the organic phase. Specifically, the claim now recites that the water added is "at least 5% by weight of the organic phase employed." The claim has been clarified, not substantively changed from what is described in paragraph [0055] of the specification. Thus, no new matter has been added in the amendment.

New Claim 21

Originally, claim 2 recited the two ranges of excess alcohol supported by paragraph [0023], a wider range of 2-100 % excess alcohol, and a "preferred" range of 5-20% excess alcohol. To avoid reciting two different ranges in the same claim, Applicants have amended claim 2 to recite only the broader range. The narrower 5-20% range is now recited in new claim 21, which depends from claim 2. Because the added limitation contained in new claim 21 was originally recited in claim 2, no new matter has been added.

New Claim 22

Originally, claim 4 recited the use of two different classes of alcohol: (1) a broader group of alcohols which are "quite soluble in the organic phase formed, but considerably less soluble in water," and (2) a narrower group within class (1) including "medium-chain to long-chain n- and iso-alcohols." To avoid reciting two different classes of alcohol in the same claim, Applicants have amended claim 4 to recite only the broader group. The use of the narrower group of "medium-chain to long-chain n- and iso-alcohols" is now recited in new claim 22, which depends from claim 4. Because the added limitation contained in new claim 22 was originally recited in claim 4, no new matter has been added.

35 USC § 102

The Office has rejected claims 1, 4, 6, 7, 8, 11-14 and 19-20 under 35 USC § 102(b) as being anticipated by Buhler (Fat Science Technology 1987, 89(14): 598-605). The Office alleges that Buhler teaches every limitation of the claims, including the use of glycerol as a possible alcohol, as recited in claims 1, 4, 6, 7, 8, 11-14 (and presumably claims 19, 20 and new claims 21 and 22). Applicants respectfully disagree. Claims 1-8, 20, 21, and 22 are clearly not anticipated by Buhler, which teaches enzymatic hydrolysis of fats, not the enzymatic alcoholysis of fats as recited in claims 1-8, 20, and 21. In fat hydrolysis, the solvent water reacts with the fat to split the fat triglyceride esters into glycerol and fatty acids. See Buhler p. 598, col. 1. Nowhere does Buhler disclose the transesterification of the triglyceride esters into different fatty acid esters, as recited in claims1-8, 20, and 21. See Buhler, entire paper.

In contrast to the process disclosed in Buhler, the transesterification process recited in claims 1-8, 20, 21, and 22 and disclosed in the specification of the present application is not hydrolysis. Rather, it is recognized in the art as alcoholysis, the solvolysis reaction wherein an alcohol (rather than water) is added to the reactant. Unlike hydrolysis, alcoholysis of a fat would lead to the production of a new fatty acid ester. To clarify the claims so that they more accurately describe the claimed process, Applicants have amended claims 1, 5, 7, and 8 to substitute the term "alcoholysis" for "hydrolysis" in the appropriate places. Similarly, the specification has been amended to substitute "alcoholysis" for "hydrolysis" in the appropriate places and to add the term "alcoholysis" as an additional descriptor when referring to the transesterification reaction recited in the claims. Because one skilled in the art would recognize that the transesterification

process as originally claimed and as described in the original as filed specification is correctly labeled alcoholysis rather than hydrolysis, these clarifying amendments add no new matter.

Because it does not teach all the limitations of the claims, Buhler does not anticipate claims 1-8, 20, 21 and 22 as currently amended. Claim 1, on which claims 2-8, 20, 21 and 22 depend, now recites "causing the lipases, as biocatalysts for alcoholysis of oil or fat and formation of fatty acid esters in a fat alcoholysis /esterification, to act on a mixture of triglycerides, water, and an alcohol soluble in oil or fat to create a reaction mixture formed in the fat alcoholysis /esterification . . ." Buhler does not disclose two recited features of the amended claims. First, Buhler does not disclose the use of lipases in a fat alcoholysis/esterification reaction producing fatty acid esters. Instead, Buhler discloses the use of lipases only in a fat hydrolysis reaction producing fatty acids and glycerol. Second, Buhler does not disclose the use of "an alcohol soluble in oil or fat" as recited in claim 1.

The Office asserts that the presence of glycerol is disclosed in Buhler, and that because any alcohol can be used in the claimed process, the use of an alcohol is anticipated by Buhler. Applicants respectfully disagree. First, claim 1 recites that the lipases must act on the alcohol and other reactants to catalyze an alcoholysis/esterification reaction. In Buhler, the glycerol is a product of the hydrolysis, and nothing suggests that the glycerol would itself could become a reactant in an alcoholysis reaction process. In fact, Buhler teaches that the glycerol must be removed as it forms to achieve 100% hydrolysis with no further addition of enzyme. See Buhler p. 601 col. 2. Clearly, Buhler does not disclose the use of glycerol as a reactant in an alcoholysis reaction, as recited in the present claims.

A second reason why the presence of glycerol in the process disclosed in Buhler does not anticipate the presence of the alcohol reactant in claims 1-8, 20, and 21 is that claim 1 recites a reaction mixture containing "an alcohol soluble in oil or fat" (emphasis added). Both Buhler and the specification of the present application clearly indicate that glycerol is water soluble, and is not soluble in oil or fat. For example, the hydrolysis process in Buhler is monitored by measuring the glycerol concentration in the water phase by refractometry, and percent glycerol in the water phase reportedly exceeded 20% as hydrolysis proceeded. See p. 599 col. 2, p. 600, Figure 1. In addition, Buhler discloses that the glycerol product was extracted continuously with the water phase. See page 601, col. 2, Figure 4, page 602, Figure 6, page 603, Figure 11.

Similarly, the present specification discloses that glycerol is water soluble, and not soluble in fat or oil. For example, paragraphs [0009] and [0014] state that the aqueous phase

contains the glycerol product and that the organic phase contains the fatty acid ester product. Paragraphs [0018] and [0019] refer to the "glycerol-containing" aqueous phase as separate from the organic phase containing free fatty acids and fatty acid esters. Paragraph [0024] contains a detailed discussion of glycerol's "extremely high" solubility in water and "very low" solubility in the hydrophobic organic phase. This results in the loss of glycerol product to the aqueous phase, which drives the chemical equilibrium to the ester product side of the reaction. The importance of these solubility differences to the chemical equilibrium is further discussed in paragraphs [0032]-[0037]. Finally, paragraph [0041] reiterates (as recited in claim1) that water soluble alcohols do not work well in the disclosed method. Because glycerol is clearly water soluble, not fat soluble, it cannot be used as an alcohol in the alcoholysis method of claim 1. Thus, all the limitations of claims 1-8, 20, 21 and 22 are not disclosed in Buhler, and Buhler does not anticipate those claims.

Further, in regard to claim 4, nothing in Buhler teaches the use of an alcohol that is "quite soluble in the organic phase formed, but is considerably less soluble in water." The Office references the presence of glycerol in the method taught by Buhler, but as discussed above, glycerol is clearly not "quite soluble in the organic phase formed," but "considerably less soluble in water." Similarly, in regard to claim 22, glycerol is not a "medium-chain to long-chain n- or iso-alcohol," as is used in the method recited in claim 22. Because Buhler does not disclose the use of the alcohols recited in claims 4 and 22, it cannot anticipate those claims.

Applicants note that of the pending claims originally reciting the alcoholysis method (1-8, 20, 21 and 22), the Office did not reject claims 2, 3, 5 or the subject matter of new claim 21 (originally contained in claim 2) as being anticipated by Buhler. Accordingly, Applicants respectfully request the Office to allow claims 2, 3, 5, and 21. In addition, in light of the amendments and arguments presented herein, Applicants respectfully request the Office to reconsider and withdraw the anticipation rejections of claims 1, 4, 6, 7, 8, 20, and 22.

In response to the anticipation rejections, Applicants have also amended claims 11-14, which were originally directed at a method of hydrolysis of fats and/or oils, to now recite the enzyme-catalyzed alcoholysis of fats and/or oils to produce fatty acid esters. These amendments also apply to previously presented claim 19, which depends from amended claim 14. As discussed in detail above, the use of the present method in which a fat or oil is reacted with an alcohol to produce a fatty acid ester (alcoholysis) is not disclosed in Buhler. Buhler discloses only the hydrolysis of a fat or oil with water to produce a fatty acid and glycerol. Therefore

claims 11-14 and 19 as amended are not anticipated by Buhler. Accordingly, Applicants respectfully request the Office to reconsider and withdraw the anticipation rejection of claims 11-14 and 19.

Petition and Fees

A Petition for a One Month Extension of Time is included with this response to make the response timely. Please charge the one month extension fee to Deposit Account No. 17-0055. No additional extension of time or fee is believed to be necessary. However, if an additional time extension is needed in this or any other response, please consider this a petition for the appropriate time extension. Similarly, if any additional fee is deemed due, please charge any additional fees due to Deposit Account No. 17-0055.

Respectfully submitted,

Dated:December 17, 2008

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SUBSTITUTE SPECIFICATION **MARKUP COPY**

A PROCESS AND DEVICE FOR OBTAINING FATTY ACID ESTERS FROM NATIVE OILS AND FATS BY THEIR ENZYMATIC HYDROLYSIS

GOVERNMENT SUPPORT STATEMENT

[0001] Not applicable.

RELATED APPLICATIONS

[0002] This application is the U.S. National Phase of PCT/EP02/06077, filed on June 4, 2002, which claims the benefit of DE 101 56 584.4, filed November 20, 2001, and EP 01 115 081.0, filed June 21, 2001.

FIELD OF THE INVENTION

[0003] ~~A process and device for obtaining fatty acids or fatty acid esters from native oils and fats by their enzymatic hydrolysis and optional simultaneous esterification with alcohols, especially n- and iso-alcohols~~The invention concerns a process and a device for obtaining fatty acids or fatty acid esters from native oils and fats by their enzymatic hydrolysis ~~and optional simultaneous~~ or esterification using alcohols (alcoholysis), especially n- and iso-alcohols.

BACKGROUND OF THE INVENTION

[0004] Enzymatic hydrolysis of oils and fats has ~~indeed~~ been known for a long time. However, high pressure hydrolysis is generally much more useful for large scale operations, ~~but has not been able to compete with hydrolysis under pressure~~ because of the enzymatic hydrolysis results in a prohibitively high amount of enzyme consumption.

[0005] The fatty acid esters from native fatty acids contained in fats and oils, and medium-chain (chain length less than 6 carbons) to long-chain (generally up to a 24-carbon chain length) n- and iso-alcohols are of high economic importance in numerous applications, especially in the lubricant field.

[0006] It is quite difficult to prepare esters of these alcohols with unsaturated fatty acids, especially oleic acid esters, by classical chemical routes such as acidic esterification. Enzymatic preparation of these esters from fatty acids and alcohols has not been carried out in the past because of the high enzyme requirement.

BRIEF DESCRIPTION OF THE INVENTION

[0007] The objective of this invention is to provide an economically feasible enzymatic process for producing fatty acids and fatty acid esters from native oils and fats, and a corresponding device. This objective is realized in the subject matter of the independent claims. Preferred further embodiments are defined in the subclaims.

[0008] The inventors have developed an economically efficient enzymatic process for hydrolyzing and alcoholyzing fats or oils, and a corresponding device to accomplish the process. They have also found that this process and device together constitute an outstanding solution to the previous problems of enzymatic hydrolysis and enzymatic production of the esters of interest (alcoholysis) in one operation.

[0009] The process of the present invention for the enzymatic preparation of fatty acid esters is characterized by the fact that lipases, as biocatalysts for the hydrolysis of oils or fats and for esterification (alcoholysis), are caused to act on a mixture of an oil or fat, water, and a fat- or oil-soluble alcohol, especially n- and/or iso-alcohols. The resulting reaction mixture is transferred to a self-discharging centrifuge in order to separate the glycerol-containing aqueous phase formed in the combined hydrolysis-esterification (alcoholysis) process from the organic phase which contains the fatty acid esters. The centrifuge is adjusted so that an intermediate layer enriched in lipase (enzyme) that forms between the aqueous and organic phase collects in the centrifuge drum. The centrifuge drum is emptied at specified times and the discharged contents are returned to the combined hydrolysis and esterification (alcoholysis) process. The contents of the drum are also available to be used in another, separate hydrolysis and esterification process (alcoholysis), in which is incorporated into the present process or just made available for a later process.

[0010] Without addition of the alcohol, enzymatic hydrolysis occurs without simultaneous esterification, and the hydrolysis makes possible an extremely economical production of free fatty acids and glycerol. The basic procedure is otherwise identical.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1a shows a schematic representation of an example of an industrial process of the present invention, designed for the case in which the resulting fatty acid esters are to be separated from the unreacted fatty acids and alcohol by distillation.

[0012] Fig. 1b shows a corresponding schematic representation of a modified process for the case in which the fatty acid esters are only separated together with the free fatty acids by distillation.

[0013] Fig. 2 shows a schematic representation of an example of an industrial hydrolysis process of the present invention, in which no esterification occurs and which is operated without addition of alcohol.

DETAILED DESCRIPTION OF THE INVENTION

[0014] In one aspect of the invention, lipases are caused to act on a mixture of an oil or fat, water, and a fat- or oil-soluble alcohol, especially n- and/or iso-alcohols. The resulting reaction mixture is transferred to a self-discharging centrifuge in order to separate the glycerol-containing aqueous phase formed in the combined hydrolysis-esterification (alcoholysis) process from the organic phase which contains the fatty acid esters. The centrifuge is adjusted so that an intermediate layer enriched in lipase (enzyme) that forms between the aqueous and organic phase collects in the centrifuge drum. The centrifuge drum is emptied at specified times and the discharged contents are returned to the combined hydrolysis and esterification (alcoholysis) process. The contents of the drum are also available to be used in another, separate hydrolysis and esterification process (alcoholysis), in which is incorporated into the present process or just made available for a later process.

[0015] Without addition of the alcohol, enzymatic hydrolysis occurs without simultaneous esterification, and the hydrolysis makes possible an extremely economical production of free fatty acids and glycerol. The basic procedure is otherwise identical.

[0016] In one embodiment, [[T]]the above-mentioned intermediate layer is enriched in the plate pack of self-desludging separators, which are suitable as self-discharging centrifuges. However, in principal ribbed inserts or other internal structures such as blades and the like may be used in an equivalent manner in place of the plate pack. It is important that the centrifuge be self-discharging, making it possible for the intermediate layer that collects in the centrifuge to be discharged from time to time by emptying the centrifuge drum.

[0017] The above-mentioned intermediate layer forms during the enzymatic fat hydrolysis even when alcohol is not added, along with the separation of the two phases that form: the glycerol-containing aqueous phase and the organic phase that contains the free fatty acids formed in the hydrolysis. The problem of this emulsion-like intermediate layer is known. ~~from~~ See "Continuous Uses of Lipases in Fat Hydrolysis," M. Bühler and Chr. Wandrey, Fat Science Technology 89, December 1987, pages 598 to 605; "Enzymische Fettspaltung" [Enzymatic Fat Hydrolysis], M. Bühler and Chr. Wandrey, Fett Wissenschaft Technologie [Fat Science and Technology] 89, No. 4, 1987, pages 156 to 164; and "Oleochemicals by Biochemical Reactions ?" M. Bühler and Chr. Wandrey, Fat Science Technology 94, No. 3, 1992, pages 82 to 94.

[0018] In "Continuous Use of Lipases in Fat Hydrolysis," oil is hydrolyzed continuously in a first stirred reactor. The reaction product, which in addition to free fatty acids contains water, glycerol, and mono- and diglycerides, unhydrolyzed oil and enzyme, is transferred to a solid wall plate centrifuge which is adjusted so that the intermediate layer between the aqueous glycerol phase and the organic phase is discharged with the organic phase. The organic phase containing the intermediate layer is delivered to a second stirred reactor, to which a fresh water/enzyme mixture is also added. The reaction product from the second reactor is again transferred to a solid wall plate centrifuge, which in this case is adjusted so that the intermediate layer is discharged with the glycerol-containing aqueous phase, so that the free fatty acids produced will be discharged without any intermediate emulsion layer. The aqueous phase is returned to the first reaction, so that the enzyme portion contained in the emulsion intermediate layer is resupplied to that process. Moreover, as is usually the case with self-discharging

centrifuges, the solids that deposit on the drum wall are discharged discontinuously when the drum is emptied.

[0019] An emulsion-like intermediate layer also forms during the phase separation in the hydrolysis carried out according to the present invention, with or without simultaneous esterification (alcoholysis). This intermediate layer contains substantial quantities of enzyme, along with the organic phase that contains the free fatty acids or their esters which are formed, and with the glycerol- containing aqueous phase. However, the objective is accomplished successfully with a centrifuge having exceptionally high capability, and an efficient and nearly loss-free recycling of the enzyme is achieved. This makes it possible to perform the fat hydrolysis with a high cleavage rate and the esterification (alcoholysis) in high yield, at high enzyme concentration and consequently short reaction time, without significant loss of enzyme.

[0020] A self-discharging centrifuge is used, preferably a self-discharging separator with a plate pack. The centrifuge is adjusted so that significant amounts of the enzyme-containing interfacial emulsion are not included in the ester-containing organic phase, nor in the aqueous phase. Instead, this emulsion accumulates in the centrifuge, preferably in the region of the separation zone in the plate pack of a separator. This is an unusual operating configuration to the extent that generally the accumulation of large quantities of an intermediate layer in the plate package during liquid/liquid phase separation is specifically avoided with the known self-discharging separators or self-desludging separators. On the contrary, one generally takes care that the smallest possible amount of this intermediate layer is produced, and it is generally discharged with the phase which is not the principal one to be recovered. This is also essentially what was done in the above publication, in which the intermediate layer was discharged with the aqueous phase from the second continuously operating solid wall centrifuge and resupplied to the first reactor. Furthermore, with self-discharging centrifuges, generally the solids that deposit on the drum wall are discharged discontinuously when the drum is emptied.

[0021] For one skilled in the art, adjustment of the centrifuge according to the present invention means applying the measures known to the art for adjusting the weir and/or adjusting the back pressure at the exit port to insure that both the organic phase and the aqueous glycerol-containing phase discharge as clear and free of emulsion as possible.

[0022] In the process of the present invention, the presence of alcohols does not result in either significantly higher enzyme consumption or substantially extended reaction time. The recovery of the esters from the reaction mixture in a simple manner is also possible.

[0023] The alcohols ~~are~~ can be added in the stoichiometrically required proportion for ester formation, but in one aspect of the invention, it is advantageous to use an excess of 2% to 100%, preferably 5% to 20%, ~~based on the stoichiometric requirement~~ as compared to the amount of alcohol stoichiometrically necessary for the complete esterification of the corresponding oils or fats. An excess of alcohol accelerates the fat ~~hydrolysis~~ alcoholysis and unexpectedly causes complete ~~hydrolysis~~ alcoholysis of all glycerides in a short time.

[0024] The glycerol formed in the reaction moves into the water phase because of its extremely high solubility in water and very low solubility in the hydrophobic organic phase. Since the medium-chain to long-chain alcohols are very poorly soluble in water but quite soluble in the organic phase, while water on the other hand is very poorly soluble in the organic phase, these alcohols are converted enzymatically to fatty acid esters according to the chemical equilibrium. This occurs either by esterification of the fatty acid derived from the fat hydrolysis with the loss of water, where this latter product migrates to the aqueous phase, or by transesterification (alcoholysis) of the oils or fats with loss of glycerol, where this latter product likewise moves into the aqueous phase. Thus, the chemical equilibrium in the organic phase lies completely on the side of the ester.

[0025] The addition of more than the stoichiometric amount of alcohol will shift the equilibrium further toward the ester side, and the rate of reaction will be increased significantly. In many cases the enzymatic fat ~~hydrolysis~~ alcoholysis of the present invention is nearly complete even with the addition of a slight excess of 5% over the stoichiometric amount of alcohol required for complete reaction of the oils or fats. This has been demonstrated for a series of n- and iso-alcohols from C8 to C24. The hydrophobic organic phase contains no mono-, di- or triglycerides. It consists solely of the fatty acid esters of the added alcohol, a small amount of the free fatty acids along with the corresponding amount of alcohol, and the excess alcohol. It is possible to remove the

excess alcohol, including the unreacted alcohol portion, and the free fatty acids from the organic phase, in order to obtain the pure esters.

[0026] The methods used to recover the pure esters depend on the nature of the fatty acids and the alcohols. For instance, if esters of C18 fatty acids and C18 alcohols are produced from corresponding oils or fats and alcohols, it is possible to remove the free C18 fatty acids along with the excess C18 alcohol from the end product of the hydrolysis/esterification (alcoholysis) reaction by distillation, because the fatty acid esters produced have a substantially lower vapor pressure than the free fatty acids and alcohols.

[0027] It is possible for the fatty acids and unreacted alcohol removed by distillation to be recycled to the combined hydrolysis/esterification (alcoholysis) reaction, so that they will likewise be converted to esters without loss. In continuous operation, then, only the stoichiometric amount of the starting oil is added, while the residual amounts of alcohol and the free fatty acids are recycled.

[0028] In some cases, the free fatty acids and the resulting esters may have approximately the same vapor pressure. This occurs, for example, in the esters of C18 fatty acids with C13 iso-alcohols. In these cases, it is possible to remove the excess amounts of C13 iso-alcohol by distillation and to separate the free C18 fatty acids from the ester/fatty acid mixture in the distillation pot by base extraction, followed by neutralization, so that these are available for recycling.

[0029] One interesting variant of fat ~~hydrolysis~~ alcoholysis with an integrated esterification or transesterification occurs with use of oils in which the fatty acids are not randomly bound to the alcohol groups of the glycerol but rather systematically , as for example the erucic acid in crambe oil. In this case, with proper selection of the ~~hydrolyzing~~ alcoholyzing enzyme, it is possible to target the production of only the erucic acid esters of the added alcohols and monoglycerides of the other fatty acids of the crambe oil, or erucic acid diglycerides and fatty acid esters of the non-erucic acids. The resulting mixtures are separated by methods known to the art, preferably by distillation. For example, it is possible to separate by distillation the mixture of erucic acid diglycerides, excess C13 iso-alcohol, and C18 fatty acid – C13 iso-alcohol ester obtained, after using a chain-length specific hydrolyzing enzyme and iso-C13 alcohol as the alcohol component.

[0030] Particularly after the separation of a component formed selectively by a specific enzyme (e.g., the C18 fatty acid – C13 iso-alcohol ester), it is advantageous to subject the mixture of substances resulting from the enzymatic ~~hydrolysis/esterification~~ alcoholysis to a subsequent, second enzymatic hydrolysis or alcoholysis process using another enzyme. This method allows one to obtain the fatty acids or fatty acid esters for which this latter enzyme exhibits selective catalytic action in the hydrolysis ~~and~~ or alcoholysis/esterification process. For example, it is possible to convert an erucic acid diglyceride, free of C18 fatty acids as explained later, to glycerol and erucic acid, or to an erucic acid ester in the presence of an alcohol, in a second enzymatic hydrolysis or alcoholysis process using an enzyme which is not chain-length specific.

[0031] The process of enzymatic fat hydrolysis or alcoholysis starting from oils and fats of the present invention thus allows the targeted production of fatty acids and fatty acid esters which previously could be obtained only at substantially higher cost. This process utilizes the known action of lipases as biocatalysts that establish a chemical equilibrium between esters, alcohols, water and acids. They act particularly on fats and oils. The latter are glycerol esters, primarily triglycerides, of medium- to long-chain and generally unbranched fatty acids. Lipases operate at the phase boundary of two-phase systems having oil or fatty acids as the hydrophobic phase and water as the hydrophilic phase, and establish a chemical equilibrium in both phases. The position of the chemical equilibrium is determined by the concentrations of the particular materials in the particular phases.

[0032] In the phase system of water and oil or native fatty acid, the water concentration is dominant in the water phase but very slight in the oil phase. As glycerol is very highly soluble in water, but hardly soluble at all in the hydrophobic phase of oil or hydrophobic fatty acids, the glycerol concentration at equilibrium must be substantially higher in the water phase than in the oil phase. Glycerol present or formed in the oil phase passes almost completely into the water phase. Native fatty acids with medium to long fatty acid chains are hydrophobic and almost insoluble in water. Their concentration in the water phase is consequently very low. On the other hand, they are quite soluble in the hydrophobic phase, and they themselves will occasionally constitute the hydrophobic phase. When lipases act on the oil at the water/oil interface, the oil is hydrolyzed with the

consumption of water to diglycerides and monoglycerides, and finally to fatty acids and glycerol.

[0033] At equilibrium, the law of mass action applies to both phases:

Water phase

$\{[\text{fatty acid}]^3 \cdot [\text{glycerol}]\} / \{[\text{triglyceride}] \cdot [\text{water}]^3\} = K$

Oil / fatty acid phase

$\{[\text{fatty acid}]^3 \cdot [\text{glycerol}]\} / \{[\text{triglyceride}] \cdot [\text{water}]^3\} = K$

[0034] For the system of reaction equations for hydrolysis of triglyceride to fatty acids:

1.) Triglyceride + H_2O $\leftrightarrow$ diglyceride + fatty acid

2.) Diglyceride + H_2O $\leftrightarrow$ monoglyceride + fatty acid

3.) Monoglyceride + H_2O $\leftrightarrow$ glycerol + fatty acid

[0035] Then, from the law of mass action:

$K_1 = \{[\text{triglyceride}] \cdot [H_2O]\} / \{[\text{diglyceride}] \cdot [\text{fatty acid}]\}$

$K_2 = \{[\text{diglyceride}] \cdot [H_2O]\} / \{[\text{monoglyceride}] \cdot [\text{fatty acid}]\}$

$K_3 = \{[\text{monoglyceride}] \cdot [H_2O]\} / \{[\text{glycerol}] \cdot [\text{fatty acid}]\}$

$K = K_1 \cdot K_2 \cdot K_3 = \{[\text{triglyceride}] \cdot [H_2O]^3\} / \{[\text{glycerol}] \cdot [\text{fatty acid}]^3\}$

[0036] The water concentration $[H_2O]$ in the organic phase is low and constant. As glycerol goes predominantly into solution in the water phase, the glycerol concentration, [glycerol], in the organic phase is also low and thus quasi-constant. Thus:

$K \cdot [\text{glycerol}] / [H_2O]^3 = K' = [\text{triglyceride}] / [\text{fatty acid}]^3$

[0037] Because of the differences in the solubilities of the individual components in the hydrophilic and hydrophobic phases, lipases hydrolyze fats and oils almost completely to glycerol and fatty acids when the water concentration in the hydrophilic

phase is high. The glycerol that is formed thus dissolves in the water, and the fatty acids initially dissolve in oil, but later form a separate hydrophobic fatty acid phase.

[0038] If the enzymatic fat ~~hydrolysis~~ alcoholysis of the present invention is carried out in the presence of added alcohols other than the glycerol contained in the fats and oils, the lipases will likewise produce a chemical equilibrium in the hydrophilic and hydrophobic phases. Exceptions to this are found for alcohols and alcohol concentrations that inhibit the activity of the enzyme, or which are incompatible with it and inactivate it. Even in this case, the position of the chemical equilibrium is determined by the distribution coefficients of the individual components between the hydrophilic and hydrophobic phases. The calculation or estimation of the chemical equilibrium distribution in two phases is naturally more complex than for simple fat or oil hydrolysis. That is particularly true for multifunctional alcohols which are water-soluble, for example trimethylolpropane and pentaerythritol.

[0039] The behavior of hydrophobic alcohols that are practically insoluble in water (both monohydric and polyhydric alcohols) is considerably easier to calculate. The medium-chain (starting with a C6 chain length) to long-chain n- and iso-alcohols of the present invention are included in this group. Such alcohols when added to a two-phase system will in practice be soluble only in the organic phase. The glycerol-containing aqueous phase will contain very little alcohol as a consequence of its low saturation concentration. Addition of such alcohols shifts the hydrolysis equilibrium toward hydrolysis because of esterification and consumption of free fatty acids. This effect is increased by the addition of excess alcohol, so that there will no longer be any tri-, di- or monoglycerides in the organic phase at equilibrium.

[0040] For such alcohols, which are quite soluble in the organic phase, the above reaction in the presence of alcohol is described as follows:

This is essentially independent of the nature of the alcohol:

Ester + H_2O $\leftrightarrow$ fatty acid + alcohol

K_{ester} = {[ester] · [H_2O]} / {[fatty acid] · [alcohol]}

In contrast to water-soluble alcohols, for alcohols which are quite soluble in the organic phase: [alcohol] is saturated in H_2O, and so is constant. Thus:

$$K' \cdot K_{ester} / [H_2O] = K'' = \{[triglyceride] \cdot [ester]\} / \{[fatty\ acid]^4 \cdot [alcohol]\}$$

[0041] The process of the present invention is carried out very successfully with alcohols whose water solubility is less than 5 wt.%, based on the aqueous phase. The yield and conversion rate decrease with increasing water solubility of the alcohol. For instance, when TMP was used as the alcohol in the process of the present invention, the conversion rate decreased by about 50%.

[0042] Some lipases, called specific lipases, are unable to hydrolyze all the fatty acid ester glyceride bonds. In particular, it is not possible for certain lipases to hydrolyze the central fatty acid bonded to the glycerol C2. Such lipases are used to target the production of monoglycerides and fatty acids, for example. If the starting oils contain certain fatty acids that are not bound in the glyceride of the oil or fat randomly, but rather are systematically distributed, then it is possible to obtain fatty acids or their esters which do not correspond to the fatty acid pattern present in the triglyceride though the use of specifically acting lipases.

[0043] For instance, it is known that long-chain fatty acids with chain lengths > 20, such as erucic acid, are always bound to the outer hydroxyl groups of glycerol in native oils and fats, and not to the central hydroxyl group. In oils rich in erucic acid such as crambe oil, for instance, with more than 60% by weight erucic acid (and about 6% by weight of fatty acids > C22), practically all the fatty acids having chain lengths > C20 are bound to the glycerol C1 and C3, while the remaining 33.33 mole-percent of C18 fatty acids are bound to the glycerol C2. In this case, a specific lipase is used to target the cleavage of only the terminal acids and produce their esters. Then, the erucic acid esters are isolated by fractional crystallization, for example. In this case, the enzyme also acts at the phase boundary layer, and it is possible for it to be recycled efficiently according to the present invention.

[0044] It is also known that the activities of particular lipases often depend on the chain length of the particular fatty acids or on the degree of and the configuration (cis,

trans, conjugated or not conjugated, etc.) of unsaturation of the fatty acids. The process of the present invention also utilizes these effects for targeted production of fatty acids or their esters. In this case, too, the enzyme catalysis occurs in the boundary layer between the hydrophilic and hydrophobic phases. Recycling of the enzyme and removal of components enable the targeted production of the desired products. This is explained with the above-mentioned example of crambe oil.

[0045] The nonspecific lipase from *Candida rugosa* cleaves long-chain fatty acids such as erucic acid considerably more slowly than C16 and C18 fatty acids. When carried out according to the present invention, the enzymatic fat hydrolysis using *Candida rugosa* lipase yields the 1,3-diglyceride of erucic acid along with the fatty acids cleaved from the glycerol C2. These latter are C18 fatty acids, and these fatty acids and their esters are separated advantageously from the diglyceride as the distillate when short-path distillation is used. It is possible to realize similar results for many other fatty acids such as omega-3- and omega-6-fatty acids by using suitable enzymes.

[0046] As noted above, the separation of the fatty acids from the reaction products of the hydrolysis reaction or, alternatively, the separation of the fatty acid esters from the reaction products of the hydrolysis or acoholysis/esterification reaction, is preferably carried out by vacuum distillation, and especially by gentle short-path distillation.. If the fatty acid esters produced have lower vapor pressure than the free fatty acids and alcohols, then the distillate contains the excess amount of n- or iso-alcohols, the unreacted alcohol, and the free fatty acids. The distillate is preferably recycled back to the hydrolysis/esterification process.

[0047] It is possible to use adsorptive separation (such as column chromatography) as an alternative to the separation of the fatty acid esters or fatty acids by distillation.

[0048] Vacuum thin-film evaporators such as falling film evaporators or short-path stills are gentler than batch distillation, and above all are operated continuously, and so their use is preferred. In any case, these distillation techniques also require a liquid residue of at least 5% to 10%, as otherwise the distillation film will break. This condition is satisfied in the present invention for both the hydrolysis and the combined hydrolysis/esterification (alcoholysis) processes.

[0049] As previously discussed, the residue from the distillation in the combined hydrolysis/esterification (alcoholysis) process contains either the desired ester, or the ester and unreacted free fatty acids. In the case of a reaction involving only hydrolysis, the distillate contains the hydrolyzed fatty acids. The pot, or distillation residue, contains the unreacted triglycerides, and is recycled back to the hydrolysis process.

[0050] The emulsion-like intermediate layer collected in the centrifuge according to the present invention, in the plate pack of a separator, according to the preferred embodiment, is discharged discontinuously by completely emptying the drum periodically, and the enzyme-containing intermediate layer obtained is reused. In this case it is convenient always to empty the drum when the discharged organic phase and/or the glycerol-containing aqueous phase begins to become turbid with the discharged intermediate layer. It is also possible to empty the drum more frequently at specified times, but this is not preferred. The fact that both the aqueous and organic phases are also discharged when the drum is emptied fully is not disadvantageous because all the phases are reused by recycling them back into the reactor. It is also possible for the drum to be emptied partially instead of fully, and these procedures should be arranged so that the intermediate layer is discharged as completely as possible.

[0051] The losses of enzyme through discharge with the aqueous and organic phases are reduced drastically in the manner shown. The discharge losses in the process of the present invention are very slight compared with time-dependent enzyme consumption (enzyme aging).

[0052] A technically interesting supplement to the invention for further reducing the enzyme loss in the separated organic phase consists of using an additional self-discharging polishing separator. This additional separator is configured immediately after the separator for the single or last hydrolysis or hydrolysis/esterification step and receives the organic phase discharged from it. This added separator is preferably a self-discharging centrifuge with a plate pack adjusted so that the solids undergoing sedimentation, the enzyme in this case, and the remnants of the aqueous phase which are still removable will separate at the drum wall. Then the quantities of enzyme centrifuged off in this manner are discharged discontinuously again and recycled back to the hydrolysis or hydrolysis/esterification (alcoholysis) process.

[0053] When a self-discharging centrifuge is used to separate the organic phase containing the fatty acid esters, it is particularly advantageous to carry out the ~~hydrolysis~~ alcoholysis reaction in loop reactors, or in other words, intermittently or batchwise, not continuously in flow reactors. Thus, for instance, a reactor is filled with oil, so that its loop is not active for circulating the oil or fat, water, alcohol and enzyme contained in the reactor. At the same time a second reactor carries out the hydrolysis or hydrolysis/esterification (alcoholysis) reaction with an active loop. Also at this time, a third reactor is being emptied through an self-discharging centrifuge, so that the reaction mixture is separated into the glycerol-containing aqueous phase, the organic phase containing the separated fatty acids or fatty acid esters, and the enzyme-containing emulsion boundary phase which forms as an intermediate layer. The emulsion boundary phase is discharged discontinuously from the separator from time to time, replenished with fresh enzyme, and returned to the reactor.

[0054] It is possible to run the reaction at a high enough enzyme concentration as to produce an unusually large phase boundary due to the circulation and the shear fields produced in the circulation pumps during the loop reactor operation. Thus it is possible to keep the amount of added water low and to obtain an aqueous phase with a considerably higher glycerol concentration than was previously possible in the hydrolysis or alcoholysis processes mentioned. In this case, there is no significant lengthening of the reaction time even with more than 30 wt.% glycerol in the discharged aqueous phase. It has not previously been considered feasible to obtain such high glycerol concentrations. Furthermore, the high glycerol concentrations will drastically reduce the loss of enzyme.

[0055] An advantage of the present invention is that the amount of added water is low, both in the case involving only the hydrolysis process, as well as for the combined hydrolysis/esterification (alcoholysis) process. In the latter case, a minimum of 5% water by weight is added, based on the organic phase, including the oil or fat and alcohol used. It is possible to add more than 200% by weight water, but that just complicates the entire process unnecessarily. In order to take advantage of the high glycerol concentration in the range of 10 to 35% by weight of the resulting glycerol-containing aqueous phase made possible in the present invention, the amount of added water should preferably be in the range of 20 to 30% by weight based on the organic phase used, and not over 50% by

weight. Even in the process involving only hydrolysis, one works preferably in the range of 5 to 200% added water based on the organic phase used, preferably 20 to 30% and maximally 50% by weight.

[0056] It is possible to run the process of the present invention at a high enzyme level without consuming much enzyme. According to the present invention, even when the enzyme activity is high, the amount of lipase added to the reactor as an effective amount is generally at least 0.01 wt.%, based on the oil or fat used. The present preferred range for the amount of lipase used in the working examples is between 0.1 and 0.5% by weight, based on the oil or fat used. This high enzyme level greatly accelerates the hydrolysis as well as the hydrolysis/esterification process. In addition, the actual enzyme consumption is very low because the enzyme is recycled, so that only fractions of the initial amounts need to be added later. In actual runs, the amounts of lipase added later were less than ten percent of the active amount placed in the reactor. One skilled in the art knows that the optimal enzyme level is selected not only for the specific enzyme, but also depends on the activity of the particular enzyme preparation. Finally, one must consider that the process becomes steadily slower as the enzyme level in the process decreases. It is also known, and has previously been stated in the publications mentioned above, that increasing the enzyme level above certain values gives no advantage with respect to the process technology or economics. It is possible for one skilled in the art to determine the optimum enzyme level for the particular starting materials with a few experiments by considering these facts.

[0057] According to the present invention, it is possible for the hydrolysis or hydrolysis/esterification (alcoholysis) reaction to be carried out in just one stage in the presence of alcohol with complete release of the glycerol, with recycling of the enzyme in this stage.

[0058] However, the hydrolysis or hydrolysis/esterification (alcoholysis) reaction of the present invention is preferably carried out in multiple stages with circulating reactors, for example in two stages. The aqueous glycerol phase obtained in the second stage is then recycled back to the first stage as the aqueous phase, and fresh water as the water aqueous phase as along with the organic phase obtained from the first stage are also

cycled to the second stage. Such a two-stage or multi-stage reaction is advantageous because the enzyme loss is further minimized.

[0059] The process of the present invention is also suitable for hydrolyzing or alcoholyzing the mono-, di-, and triglycerides from soap stock obtained from the alkali refining of feed oils, and converting them to fatty acid esters. For this purpose it is preferable to release the fatty acids bound in the soaps by adding acid before the hydrolysis/alcoholysis/esterification.

[0060] Although [[T]]the invention will now be exemplified using working examples and the accompanying drawings, it is to be understood that the invention is not limited to the specific embodiments set forth and exemplified here.

~~Fig. 1a shows a schematic representation of an example of an industrial process of the present invention, designed for the case in which the resulting fatty acid esters are to be separated from the unreacted fatty acids and alcohol by distillation;~~

~~Fig. 1b shows a corresponding schematic representation of a modified process for the case in which the fatty acid esters are only separated together with the free fatty acids by distillation, and~~

~~Fig. 2 shows a schematic representation of an example of an industrial hydrolysis process of the present invention, in which no esterification occurs and which is operated without addition of alcohol.~~

[0061] Figures 1a and 1b show a configuration of the present invention for a two-stage combined fat hydrolysis/fatty acid ester formation which is suitable for realizing the features noted on a production scale. Two process stages 1 and 2 are provided, each of which comprises three circulation loop reactors. As already discussed above, the reactors are operated intermittently: filling, reaction phase, emptying phase. The circulation loop for each of the three reactors in a stage is furnished with a centrifugal pump indicated in the drawing, and preceded by a heat exchanger. The reactors are stainless steel vessels, for instance, and are equipped with stirrers. Also, a separator in the form of an self-discharging plate separator is provided for each stage.

[0062] The exit port of the separator for the second hydrolysis stage, from which the organic phase is discharged with the fatty acid esters, is connected to a vacuum short-path still, in which a short-path distillation is effected to separate the free fatty acids and the

alcohols from the fatty acid esters formed, for the case presented above in which the latter have a lower vapor pressure than the former.

[0063] The residue from the distillation will contain the desired esters if the unreacted free fatty acids and the alcohol are more volatile than the desired fatty acid ester end product. This corresponds to the process shown in Figure 1a.

[0064] If, instead, only the alcohol is obtained as the distillate, as in Figure 1b, the residue from the distillation contains both the esters and the free fatty acids. The free fatty acids are neutralized in a separator by adding an alkaline solution such as sodium hydroxide, and then are separated from the fatty acid esters as the heavier soap phase by centrifugation. The soap phase is cleaved to provide fatty acids and salts by a known method, e.g., in a second centrifuge after addition of an acid such as sulfuric acid, and the fatty acids are returned to the first stage of the reaction.

[0065] The reactor is charged with a buffer solution, the triglyceride to be hydrolyzed, the particular ester-forming alcohol, and the enzyme, i.e., lipase. More enzyme is obtained in each time the separator is emptied intermittently, and is returned to the reactor of the same stage that is being filled, with each particular separator routed to a particular reactor. Thus the enzyme remains in circulation in one stage, along with the discharged proportions of free fatty acids, unhydrolyzed triglycerides, etc. This prevents the partial mixing of starting materials of different quality from the two stages. This also reduces the risk of reverse reactions. Finally, it should also be mentioned that a glycerol solution is removed from the first-stage separator as the separated heavier liquid phase and is ready for further processing. The glycerol solution from the second-stage separator is returned to the first-stage reactor that is being filled, as shown in the figures.

[0066] A slightly acidic standard solution selected and adjusted according to the conditions specified by the enzyme producer for use as the buffer solution. An aqueous solution with sodium acetate and acetic acid, adjusted to be slightly acidic, is used in the working examples. The optimal pH of the buffer solution is adjusted for the particular enzyme.

[0067] This also applies for the process control temperature. Temperatures between 25 °C and 45 °C have been tested in experiments. Here it should be noted that the temperature is slightly elevated in general because of the exothermic reaction. In the

cases tested, though, it was easily possible to ensure that the aqueous and organic phases were kept liquid and that no crystallization occurred in the liquid phases.

[0068] In principle, it is possible for any type of enzyme presented in the above-mentioned Bühler and Wandery publications to be used. Various *Candida rugosa* enzymes were tested thoroughly. It is also suitable to use enzymes obtained from oil seeds in the process of the present invention (e.g., from castor oil). They will naturally have the advantage of a particularly selective activity for certain fatty acids. There has been scant use of such enzymes for fat hydrolysis, however, and they are generally more costly than other enzymes prepared by industrial fermentation of yeasts, molds, bacteria and the like.

[0069] Oleyl alcohol and stearyl alcohol were tested as the n-alcohols in the experimental series. Experimental series were carried out with iso-C8, iso-C10, iso-C13, iso-C16, iso-C18, iso-C20 and iso-C24 as iso-alcohols.

[0070] The esters were prepared from high-oleic sunflower oil. Depending on the application, it is possible to start with dewaxed and refined, or crude oil or fat.

[0071] It is also possible to carry out the invention with less available and certain longer-chain alcohols, from greater than C26 up to C36, and with other oils and fats.

Examples

1. Enzymatic esterification

[0072] An 80 liter stirrer vessel was charged with 20 kg of dewaxed and refined high-oleic sunflower oil 90plus®, and 22.3 kg (10% stoichiometric excess) of Isofol 20 (C20 alcohol from Fuchs Petrolub), 10.6 kg buffer solution (0.1 N sodium acetate/acetic acid, pH 4.6) and 40 g of OF Enzyme 360 (from the Meito Sangyo company) was blended in. This mixture was circulated with a centrifugal pump for 3 hours at about 40 °C.

[0073] Then the combined phase mixture was moved directly by gravity feed at a rate of about 30 kg/hr to a plate centrifuge (SA 1-01, Westfalia Separator AG, Oelde) and separated continuously. The acid value of the discharged organic phase, as determined by titration with 0.1 N KOH in alcoholic solution, according to DIN 53169 and DIN 53402, passed through a maximum of about 55, and decreased to about 15 at the end.

[0074] The enzyme was discharged from the centrifuge together with small amounts of the organic phase and glycerol-water. It showed hardly any loss of activity and could be reused.

[0075] A clear oil-ester phase and a clear glycerol solution as the aqueous phase were taken from the centrifuge. The aqueous phase contained 17% by weight of glycerol as expected.

[0076] No tri-, di- or monoglyceride could be detected in the oil-ester phase by thin-layer chromatography. After a final vacuum distillation of a 4 kg aliquot, a residue of the corresponding C20 iso-ester was obtained in 95% yield, based on the amount of oil used.

[0077] The distillate contained the excess amount and the residual unreacted portions of iso-alcohol and free fatty acids.

[0078] In a laboratory experiment, a stoichiometric mixture of oil, iso-alcohol and buffer solution was added to part of the distillate, so that the alcohol stoichiometric excess, based on the new amount of oil used, was again 10%.

[0079] The yield likewise reached 95% over the same time and with the same amount of enzyme. Here, too, comparable quantities of unreacted iso-alcohol and free fatty acids could be distilled out of the organic phase that was centrifuged off after termination of the reaction, so that it was possible to circulate the distillate without loss. It was also shown that the enzyme used (OF 360) catalyzes the esterification of free fatty acids and iso-alcohol in the presence of an aqueous phase.

[0080] The effectiveness of the process of the present invention was further tested for the n-alcohols oleyl alcohol and stearyl alcohol. For example, an experiment was carried out with equivalent success using oleyl alchol (MW = 268.49) and the lipase used in the example above. Iso-C8, iso-C10, iso-C13, iso-C16, iso-C18, iso-C20 and iso-C24 were used as iso-alcohols. Experiments were also done successfully with crambe oil, whereby the other possibilities presented above were demonstrated. A mixture of branched C16/C18 fatty alcohols (MW = 286) was also converted successfully with the lipase above according to the present invention.

[0081] In principle the process of the present invention is also applicable to synthetic fatty acid esters, such as synthetic triglycerides and other polyol esters.

2. Enzymatic hydrolysis

[0082] Figure 2 shows clearly that the process control for the process involving only fat hydrolysis without simultaneous esterification by addition of alcohol does not differ in the essential points from the foregoing, for example the process control presented above in Figures 1a and 1b. Possible mass throughputs are reported for examples, but the process can be and has been also carried out successfully with other quantities. Therefore only the distinguishing part of the process is explained. The statements above about possible temperature ranges and handling of the enzyme apply equally.

[0083] Thus the exit port of the second hydrolysis stage separator, from which the organic phase with the fatty acids (instead of the fatty acid ester) is discharged, is again connected to a vacuum short-path distillation system in which short-path distillation is used to separate the free fatty acids. The residue from the distillation was sent to a stirred crystallizer in which the waxes crystallized. In connection with that, the residual oil in which the waxes and other higher-boiling components had crystallized out as solids was pumped into a filter assembly, where it was freed of those concomitants. The oil purified in that manner was then returned to the first stage for hydrolysis.

[0084] A hydrolysis of high-oleic sunflower oil was carried out. After a starting phase, 30.0 kg of a crude, unrefined high-oleic sunflower oil 90 plus (registered trademark) from the Dr. Frische GmbH company, having an acid value of 4 as determined by titration with alkaline potassium hydroxide according to DIN 53169 and DIN 53402, was charged to one of the first-stage reactors along with 7.0 kg of a buffer solution consisting of a 12% glycerol/water solution buffered with 3.0 g sodium acetate. The charged mixture of oil and buffer solution was circulated by means of a centrifugal pump with stirring and maintained at 35 – 40 °C. Next, 2 kg of enzyme-enriched discharge product from the first separator stage of the start-up phase was added, along with 3 g fresh lipase from *Candida rugosa* (lipase in the form of a powdered solid from Meito Sangyo, Japan, 360,000 units/gram) which had also been used in the start-up phase. After 60 minutes reaction time with stirring and then being allowed to settle, the reaction mixture was separated in the first stage separator (self-discharging plate centrifuge SA1-01 from Westfalia Separator AG Co.) into an organic phase containing

fatty acids and an aqueous glycerol phase. The reactor content was fed to the separator at 40 kg/hour, and the centrifuge drum was completely emptied hydraulically every 15 minutes. The discharge product from the drum was transferred to the particular first-stage reactor that was being filled. The second-stage reactor to be filled received, along with the oleic acid-containing organic phase from the first stage separator, 7.0 kg of the above-described buffer solution, 2 kg of the discharge product from the second-stage separator from the start-up phase, and replenished with 5 g fresh lipase of the type noted above. Otherwise, this process was carried out as in the first-stage fat hydrolysis, with the result that the lighter phase discharged from the second-stage separator was a clear crude oleic acid phase with an acid value of 184, corresponding to a 93% conversion for the hydrolysis (calculated as the measured acid value divided by the theoretical acid value for this mixture). The heavy phase separated by the centrifuge constituted a 12% by weight solution of glycerol in aqueous buffer solution. The crude fatty acid thus obtained in the second stage was collected in a 200 liter vessel serving as an intermediate container. This was subjected to short-path distillation in a short-path vacuum still, Type KD, from UIC Co., Alzenau-Hörstein, with an initial degassing stage to separate any traces of water. For example, in a distillation at 191 °C and system pressure of 0.014 mbar, about 8% by weight of the fatty acid used came out continuously under these conditions as a residue of non-boiling components. The other 92% by weight of the crude product distilled as oleic acid having an acid value of 199-200, with only the slightest amounts of minor constituents in the form of fatty acids with a lower vapor pressure. This was consistent with the theoretically calculated acid value, within the accuracy of the measurement. The value was somewhat higher than the theoretical acid value because of the small proportion of more volatile, shorter fatty acids.

[0085] The residual product was dewaxed, in which the higher fatty acids (chain lengths of C22 and higher), waxes, and other higher-boiling components contained in the oil crystallized out as solids and were separated by filtration. The filtered residual product was returned to the hydrolysis process at the first stage by a pump as indicated in Figure 1.

[0086] Some other examples were run in the same manner, including some with higher proportions of enzyme and correspondingly shorter hydrolysis times; with beef

tallow, and with crambe oil. In the example with crambe oil, which contains 60% erucic acid, a mixture of the non-specific enzyme OF 360 noted above and the 1,3-specific enzyme Novozym 388 was used. The latter specifically hydrolyzes off the fatty acids bound to the 1- and 3-positions of the triglyceride structure, erucic acid in this case.

~~Summary~~

ABSTRACT

A ~~P~~process and device for obtaining fatty acids or fatty acid esters from native oils and fats by their enzymatic hydrolysis and optional simultaneous esterification with alcohols, especially n- and iso-alcohols, is disclosed. ~~Process and device for obtaining fatty acids or fatty acid esters from native oils and fats, in which lipases, as b~~Biocatalysts for hydrolysis of oils or fats, are caused to act on a mixture of an oil or fat, water, and optionally an oil- or fat-soluble alcohol to hydrolyze or alcoholyze the oil or fat. ~~and optionally to form an ester, where t~~The reaction mixture thus formed is placed in an self-discharging centrifuge for separation into a glycerol-containing aqueous phase and an organic phase, the centrifuge is adjusted so that a lipase-enriched intermediate phase collects in the centrifuge between the aqueous that is drained off and the organic phase that is drained off, and the centrifuge is emptied at specified times and the discharged drum contents from the centrifuge are returned to the combined hydrolysis or optionally combined alcoholysis ~~hydrolysis/esterification~~ process.